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RESEARCH ARTICLE

PREVALENCE AND RISK FACTORS ASSOCIATED WITH VULVOVAGINAL CANDIDIASIS DURING PREGNANCY IN SANA'A, YEMEN

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Abstract

Background and objective: Women at pregnancy are further susceptible to vaginal colonization and yeast infection. The responsibility of *Candida* colonization in the incidence of preterm birth is correctly established. Knowing regional epidemiology and identifying risk factors of preterm birth are important for management and preventive strategies. The aim of the study was to reveal the prevalence of *Candida* species in vaginal swabs of pregnant women and determine odds ratio of risks for vulvovaginal candidiasis (VVC).

Methods: Pregnant women attendance routine antenatal visits in Al-Olify –family Center in Sana'a city were registered into a cross-sectional study carried out from June 2018 to March 2019. The laboratory works were carried out in the National Center of Public Laboratories (NCPHL). Samples of vaginal swabs were taken from contributors after obtaining oral consent. Data of demographic, clinical and risk factors were collected in a pre-designed questionnaire.

Results: A total of 190 pregnant women are included. The rate of VVC was 51.6%. *Candida albicans* accounted for 39.5% and non-*Candida albicans* accounted for 12.1% of isolates, mainly *C. glabrata* (4.7%), *C. lipolytica* (3.2%), and *C. famata* (2.1%). When VVC risk factors were considered, there were significant risk factors with the age group 20-24 years (61%, odds ratio (OR)=1.8), first trimester of pregnancy (61.1%, OR=1.7), Multipara of parity (61.8%, OR=1.9), low socioeconomic level (60.1%, OR=2.4), and illiteracy (68%, OR=2.2). When clinical symptoms were considered, only 86.2% of affected females had clinical signs of VVC.

Conclusions: There is a high probable rate of VVC is found among pregnant women in Yemen, undiagnosed and unnoticed; and this highlights the need for health authorities to develop strategies for diagnosing VVC, including vaginal swabs for candidiasis as a routine procedure for all pregnant women. This study also revealed a steady increase in time with a non-*C. albicans* species prevalence rate. VVC syndrome management guidelines in Yemen should be revised to include a special protocol for pregnant women.

Keywords: *Candida*, Epidemiology, Pregnancy, Sana'a, Vulvovaginal candidiasis, VVC, Yemen.

INTRODUCTION

VVC is produced by overgrowth of Candida yeasts, especially *Candida albicans*, which is an essential component of vaginal flora[1]. VVC symptoms comprise vaginal discharge, pain, itching and swelling of vulvar. In addition, vulgar erythematosus and edema with obstruction are common results. Typical vaginal secretions in VVC are described as cottage cheese-like in character[2]. It is possible that 75.0% of women may experience VVC during their lifetime[3]. Microbiological and epidemiological studies indicate that intrauterine infection accompanies up to 40% of preterm births[4]. The *Candida* colonization role was confirmed in the preterm birth occurrence[5], as the *Candida* were isolated from amniotic fluid in spontaneous women preterm birth[5,6]. *Candida* has been found to increase metalloproteinase 9 production by chorioamniotic membranes[6]. Metalloproteinase nine is a protein that remodels connective tissue and has an essential role in

the origin of premature labor and early rupture of membranes[7]. Also, the mother's vulvovaginal candidiasis is a most important risk factor for neonatal *Candida* colonization[8]. There is evidence that eliminating *Candida* species during pregnancy may reduce the risk of spontaneous premature birth[9]. In epidemiology, VVC is the next most common cause of vaginosis worldwide, following a bacterial infection. In non-pregnant women, the risk of VVC is about 20%, but it increases by 30% during pregnancy[10]. Usually (90%) of infections due to *Candida albicans* are undisruptive and do not cause any symptoms. However, vaginal or vulvar infection may be linked with symptoms and signs for instance severe itching, pain, pruritis, irritation, bad odor, dyspareunia, and dysuria and burning in urination[10]. In the Arabian Peninsula, the epidemic of VVC infection varies in diverse countries, even from regions inside the same country[11-13]. Thus, local studies are essential to obtain related epidemiological data and features of Candida sensitivity to antifungal drugs with the purpose of help manage and treat patients with *Candida* infection.

Regarding the causative species of VVC, some researchers report an increased rate of non-*Candida albicans* species, particularly *C. glabrata*, *C. krusei* and *C. parapsilosis*[14]. Some researchers have also found that the incidence of *C. albicans* infection decreases and other *Candida* types including *C. glabrata. C tropicalis, C. kefyr, C. parapsilosis, C. africana, C. famata, C. dubliniensis, C. guilliermondii and C. lusitaniae* associated mainly with vulvovaginitis from patients with immunodeficiency or in pregnant women[10]. In Yemen, vaginitis is one of the most common conditions for seeking medical care. In the city of Sana'a, vaginal infections have spread in Yemen among 37.6% of women of childbearing age, with VVC associated significantly with less than 25 years of age and use of intrauterine contraceptives[15], also in Hadramaut three hundred and seventy two (39.2%) of the 950 pregnant women suffered from abnormal vaginal discharge and were positive for bacterial vaginosis[16]. Pregnant women are more likely to experience vaginal colonization and *Candida* infection. The responsibility of *Candida* colonization in the happening of preterm birth is well established. Knowing local epidemiology and identifying risk factors for preterm birth are important for prevention and management strategies, so the purpose of the current study was to determine the prevalence of *Candida* species in the vaginal swabs of pregnant women and to identify the risk factors associated with VVC.

SUBJECTS AND METHODS

Pregnant women attending routine antenatal visits in Al-Olify –family Center in Sana'a city were enrolled into a cross-sectional study carried out from June 2018 to March 2019. Inclusion criteria for subject selection were healthy individuals with no systemic disease. In addition, pregnant women who currently taking antifungal, steroids, antibiotics, or immunosuppressive drugs in the past 6 months were excluded. The sample included 190 pregnant mothers. All pregnant women was examined clinically by specialist and vaginal swabs were taken. The vaginal swabs were sent to the National Center of Public Laboratories (NCPHL) where the laboratory works were carried out. Vaginal swabs were sampled from the participants after oral approval. Smears were inoculated in chloramphenicol Sabouraud's glucose agar and incubated at 37°C for 24 to 48 hours under aerobic conditions for performing fungal cultivation[17]. *Candida* species were identified by culturing HiCrome differential Agar at 35°C for 48 hours to produce species specific colors. Data of demographic, clinical and risk factors were collected in a pre-designed questionnaire.

Data analysis

The data was statistically analyzed using EPI-Info version 6. The difference in the distribution of *Candida* types among groups was based on a comparison of repeat distributions by chi-square test. The odds ratio associated with VVC risk factors was performed by 2x2 tables to obtain an *OR*, 95% *CI*, Chi squared and *p* value by uncorrected static tests where the value of $p<0.05$ was considered significant.

Ethical approval

We obtained written consent in all cases. Approval was obtained from the participants prior to collection of samples. The study proposal was evaluated and approved by the Ethics Committee, Faculty of Medicine and Health Sciences, University of Sana'a.

RESULTS

A total of 190 pregnant women were included, ranging in age from 17-37 years, with mean±SD of 25.4±4.9 years, most of the women were in the age group 20 -24 years (37.9%) and 25-29 years (30.5%) (Table 1). The prevalence of VVC was 51.6%. *C. albicans* accounted for 39.5% and non-*Candida albicans* accounted for 12.1% of isolates, mainly *C. glabrata* (4.7%), *C. lipolytica* (3.2%), and *C. famata* (2.1%).

Table 1: The distribution of pregnant women according to their age.

Age groups	Case n=190 pregnant women	
	No.	%
< 20 years	18	9.5
20 -24 years	72	37.9
25-29 years	58	30.5
30-34 years	28	14.7
≥ 35	14	7.4
Mean age	25.4 years	
S. D	4.9 years	
Mode	25 years	
Median	25 years	
Max	39 years	
Min	17 years	
Total	95 %	

When VVC risk factors were considered, there were significant risk factors with the age group 20-24 years (61%, *OR*=1.8), first trimester of pregnancy (61.1%, OR=1.7), Multipara of parity (61.8%, *OR*=1.9), low

socioeconomic level (60.4%, *OR*=2.4), and illiteracy (68%, *OR*=2.2). When clinical symptoms were considered, only 86.2% of affected females had clinical signs of VVC. Subgroup prevalence of vulvovaginal candidiasis is presented in Table 2. Younger women, <20 years had a somewhat lower prevalence (33.3%) of vulvovaginal candidiasis, while in the 20-24 years and older group, the prevalence was between 41.3% and 71.4%. The adjusted odds ratio showed that vulvovvaginitis was not significantly associated with older age (*p=0.*12). The prevalence of vulvovaginal candidiasis varied with gestational trimester, parity, education and sociodemographic level. Women who are illiterate were more affected than those patients with primary school education and above. Similarly, vulvovaginal candidiasis was higher among low level of sociodemographic. Also vulvovaginal candidiasis was higher among multipara mothers (61.8%) compared to nulliparous (38.5%) or paucipares (54.4%) study subjects (Table 2). Table 4 shows the prevalence of *Candida* species according to presence of symptoms. The *Candida* was isolated in most pregnant women who had symptoms of *Candida* VV (86.2%), while only 22.3% was isolated in pregnant women who had no symptoms of *Candida* VV. There was a higher rate of isolated *albicans Candida* in women with symptoms (73.6%) compared to 10.7% in women without symptoms. On the other hand, the percentage of non-*Candida albicans* in symptomatic women (11.7%) is almost similar to 12.6% in asymptomatic women.

Table 2: The risk factors of VV Candidiasis among pregnant women in Sana'a city.

Characters	Positive VVC n=98		OR	CI	X^2	*p*
	No	%				
Age group						
< 20 years n=18	8	33.3	0.7	0.27-1.9	0.4	0.52
20-24years n=72	44	61	1.8	1.0-3.4	4.2	0.04
25-29 years n=58	24	41.3	0.5	0.29-1.0	3.5	0.06
30-34 years n=28	12	42.8	0.66	0.2-1.4	1.0	0.3
> 35 years n=14	10	71.4	2.5	0.7-8	2.3	0.12
Gestational trimester n=190						
First n= 54	33	61.1	1.7	0.9-3.2	2.7	0.09
Second n=78	36	46.2	0.7	0.3-1.2	1.5	0.21
Third n=58	29	50	0.91	0.4-1.6	0.08	0.77
Parity						
Nulliparous (0 birth) n=65	25	38.5	0.4	0.21-0.8	6.8	0.009
Paucipares (1-2 birth) n=57	31	54.4	1.2	0.6-2.1	0.25	0.6
Multipara (>2 birth) n= 68	42	61.8	1.9	1.03-3.5	4.4	0.03
Sociodemographic						
Low n= 111	67	60.4	2..4	1.3 -4.2	8.2	0.004
Intermediate n=79	31	34.4	0.4	0.2-0.76	8.2	0.004
Educational status						
Illiterate n=25	17	68	2.2	1-5.3	3.1	0.07
Primary school n=32	19	59.4	1.5	0.9-3.1	0.9	0.33
Secondary school n=65	35	53.8	1.1	0.6-8	0.2	0.65
University n=68	27	39.7	0.47	0.25-0.8	5.9	0.01
VVC clinical signs						
Yes n=87	75	86.2	21.7	10-46	77	<0.0001
No n= 103	23	22.3	0.04	0.02-0.09	77	<0.0001

OR=Odds ratio, CI= confidence interval, X^2=Chi square, *p*= *p* value.

DISCUSSION

Information on the prevalence of VVC in Yemen is not known. Unfortunately, VVC is not a reportable disease and diseases are regularly diagnosed by sign and symptoms without supporting laboratory diagnosis. The same as a result, the spectrum of yeasts involved in causing the disease is unknown in Yemen. The prevalence of VVC among pregnant women is also unknown in Yemen, but it is known worldwide that it is the second most common infection in the vaginal vulva part in women with symptoms as it represents about 17% to 42.%[18-20]. Even though the prevalence of infection in the current study among pregnant women ranging in age from 17-37 years, with mean±SD of 25.4±4.9 years was 51.6%, slightly higher than the reported range,[18-20], it was higher than the prevalence rates reported by Ahmed and others in India among pregnant and non-pregnant women[19] and Olowe *et al.*, in Nigaria among pregnant women[20], however lower prevalence was reported by ERylander *et al.*, among sexually active young women and association with orogenital sex[21]. Differences in rates can be explained by identifying differences in the sociodemographic characteristics and immunity status of patients[22], treatment of patients with extensive antibiotics, immunosuppressive drugs[23] and hormonal effects[24] as several of the factors for differences in prevalence and/or recurrent VVC between studies. Age, level of education, pregnancy stages, parity and socio-demographic status were investigated as potential risk factors for vulvovaginitis in the present study. There

was no strong evidence for the association between level of education, gestational trimester, and prevalence of VVC. Among the sociodemographic characteristics, age appears to be an important factor in the incidence of VVC in general as the rate of VVC was 61% with OR=1.8, 95% CI=1-3.9, *P*=0.04 in the 20- 24 years age group (Table 2). The current result was similar with a preceding study by Sobel *et al.*,[25], they showed that VVC is rare in puberty (the first appearance of menstruation), but its frequency increases with the end of the second decade of life (10-19 years) and reaches its peak in the third (20-29 years) and the fourth decade (30-39 years old).

Table 3: The frequency of different species of Candida isolated from pregnant women.

Micro-organism species	Pregnant women	
	No.	%
Candida spp	98	51.6
Candida albicans	75	39.5
Non-*Candida albicans* spp	23	12.1
C. globrata	9	4.7
C. dubliniensis	2	1.1
C. rugosa	1	0.52
C. lipolytica	6	3.2
C. famata	4	2.1

Although in the current study, the *odds ratio* detected for the 20-24 age group was significant; the association of older age groups and vaginal candidiasis was not statistically significant (Table 2); this may point toward that the association was affected by other variables. The incidence was more in pregnant women who are illiterate (68%) than in patients with university education (39.7%). Current results were consistent with those of Rathod *et al.*, in India[26], but in disagreement to the conclusion of Vadav and Prakash[27]. Among the six *Candida* species isolated in this study, the isolated rate for *C. albicans* was 39.5% , 9 (4.7%) for *C. globrata*, 2 (1.1%) for *C. dubliniensis*, 1 (0.52%) for *C. rugosa*, 6 (3.2%) for *C. lipolytica* and for *C. famata* 4 (2.1%) (Table 3). The results of the current study of *C. albicans* as the predominant species were reliable with similar previous studies[25,28]. This is evidenced by the current study in which the isolation rate of non-*albicans Candida* species was 12.1%. By comparison, more recovery rates for non-*albicans Candida* species were reported at 41.4% in India[29] 31.7% in Belgium[29] and 19.8% in the United States[28]. Also higher recovery rates of 53.1%, 65.0% and 57.5% of *non-albicans Candida* species have been reported in studies carried out in India[19], Egypt[31] and Iran[32], correspondingly. The recovery rate is 4.7% for *C. globrata*, 1.1% for *C. dubliniensis*, 0.52% for *C. rugosa* and 3.2% for *C. lipolytica* and *C. famata* 2.1% similar to what Trama *et al.*,[28]. Studies of Sobel *et al.*,[25], Nergessie[33], Sobel *et al.*,[34], they were discovered that *C. glabrata* was the predominant yeast among *non-albicans Candida* species. Bauters *et al.*, research[30] showed that *C. glabrata* was the most common *non-albicans Candida* species (16.3%), followed by *C. parapsilosis* (8.9%), *C. humicola* (1.6%), *C. krusei* (0.8%), and *C. lusitaniae* (0.8%).

Table 4: The prevalence of Candida species according to presence of symptoms.

Micro-organism species	Asymptomatic n=103		Symptomatic n=87	
	No.	%	No.	%
Candida spp	23	22.3	75	86.2
C. albicans	11	10.7	64	73.6
Non-*Candida albicans*	12	11.7	11	12.6
C. globrata	6	5.8	3	3.4
C. dubliniensis	2	1.9	0	0
C. rugosa	1	0.97	0	0
C. lipolytica	1	0.97	5	5.7
C. famata	2	1.9	2	2.3
Total	23	22.3	75	86.2

Hassanvand *et al.*,[35] *C. albicans* proved to be the most common isolated species followed by *C. glabrata*, *C. tropicalis*, and *C. parapsilosis*. The significance of this result can be explained with caution that *C. globrata* may replace *C. albicans* under selective pressure of fluconazole.

CONCLUSIONS

There is a high probable rate of VVC among pregnant women in Yemen, and this highlights the need for health authorities to develop strategies to diagnose VVC, including vaginal swabs for candidiasis as a routine procedure for all pregnant women. This study also revealed a steady increase in time with a non-*C. albicans* species prevalence rate. The guidelines for managing VVC syndrome in Yemen should be revised to include a special protocol for pregnant women.

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AUTHOR'S CONTRIBUTION

Al-Rukeimi ADA: clinical work, writing. **Al-Hatami SMM:** methodology, investigation. **AL-Danany DA:** formal analysis, data curation, conceptualization. **Al-Shamahy HA:** supervision, review. **Al Rukeimi RAA:** methodology, formal analysis. All authors revised the article and approved the final version.

DATA AVAILABILITY

Data will be made available on request.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

REFERENCES

1. Van Schalkwyk J, Yudin MH, Infectious Disease Committee. Vulvovaginitis: screening for and management of trichomoniasis, vulvovaginal candidiasis, and bacterial vaginosis. J Obstet Gynaecol Can. 2015; 37(3):266–74.
2. Paladine HL, Desai UA. Vaginitis: diagnosis and treatment. Am Fam Physician 2018; 97(5):321–9.
3. Achkar JM, Fries BC. Candida infections of the genitourinary tract. Clin Micr Rev 2010; 23(2):253–73. *https://doi.org/10.1128/CMR.00076-09*
4. Goldenberg RL, Culhane JF, *et al*. Epidemiology and causes of preterm birth. Lancet 2008; 371:75-84. *http://dx.doi.org/10.1016/S0140-6736(08)60074-4*
5. Ali GY, Algohary EH, Rashed KA, *et al*. AA. Prevalence of Candida colonization in preterm newborns and VLBW in neonatal intensive care unit: role of maternal colonization as a risk factor in transmission of disease. J Matern Fetal Neonatal Med 2012; 25:789-95. *http://dx.doi.org/10.3109/14767058.2011.622005*
6. Beghini J, Linhares IM, Giraldo PC, *et al*. Differential expression of lactic acid isomers, extracellular matrix metalloproteinase inducer, and matrix metalloproteinase-8 in vaginal fluid from women with vaginal disorders. BJOG 2015; 122:1580-5. *http://dx.doi.org/10.1111/1471-0528.13072*
7. Lal CV, Xu X, Jackson P, *et al*. Ureaplasma infection mediated release of matrix metalloproteinase-9 and PGP - a novel mechanism of preterm rupture of membranes and chorioamnionitis. Pediatr Res 2016; 81:75-7. *http://dx.doi.org/10.1038/pr.2016176*
8. Abdallah Y, Kaddu-Mulindwa D, Nankunda J, Musoke PM. Prevalence and immediate outcome of Candida colonized preterm neonates admitted to Special Care Unit of Mulago Hospital, Kampala Uganda. Afr Health Sci 2015; 15:197-205. *http://dx.doi.org/10.4314/ahs.v15i1.26*
9. Roberts CL, Morris JM, Rickard KR, *et al*. Protocol for a randomised controlled trial of treatment of asymptomatic Candidiasis for the prevention of preterm birth. BMC Pregnancy Childbirth 2011; 11:19. *http://dx.doi.org/10.1186/1471-2393-11-19*
10. Goncalves B, Ferreira C, Alves CT, *et al*. Vulvovaginal candidiasis: epidemiology, microbiology and risk factors. Crit Rev Microbiol 2016; 42:905-27. *http://dx.doi.org/10.3109/1040841x.2015.1091805*
11. Alsharifi EA. Epidemiology of vaginal candidiasis among pregnant women attending Tikrit teaching hospital/Iraq. J Fac Med Baghdad 2017; 59(4):1-5. *https://doi.org/10.32007/med.1936/jfacmedbagdad.v59i4.10*
12. Altayyar IA, Alsanosi AS, Osman NA. Prevalence of vaginal candidiasis among pregnant women attending different gynecological clinic at South Libya. European J Exp Biol 2016;6(3):25-29.
13. Al-akeel R A, El-Kersh T A, Al-Sheik Y A, AlAhmady Z Z. Prevalence and comparison for detection methods of Candida species in vaginal specimens from pregnant and non pregnant Saudi women. African J Microbiol Res 2013; 7(1):56-65. *https://doi.org/10.5897/AJMR12.1979*
14. Paulitsch A, Weger W, *et al*. A 5-year (2000—2004) epidemiological survey of Candida and non-Candida yeast species causing vulvovaginal candidiasis in Graz, Austria. Mycoses 2006; 49:471-5. *http://dx.doi.org/10.1111/j.1439-0507.2006.01284.x*
15. Abdul-Aziz M, Mahdy MAK, Abdul-Ghani R, *et al*. Bacterial vaginosis, vulvovaginal candidiasis and trichomonal vaginitis among reproductive-aged women seeking primary healthcare in Sana'a city, Yemen. BMC Infect Dis. 2019; 19(1):879. *http://dx.doi.org/10.1186/s12879-019-4549-3*
16. Wedad M. Al-Haik. Ahmed M Al-Haddad. Bacterial vaginosis among pregnant women in Hadhramout-Yemen. AUST J 2017; 16(7):23-33.
17. Cheesbrough M. Medical Laboratory Manual for Tropical countries. Published by Tropical Health Technology Ltd. 2000, England
18. Anderson MR, Klink K, Cohrssen A. Evaluation of vaginal complaints. JAMA 2004; 291:1368-79.
19. Ahmad A, Khan AU. Prevalence of Candida species and potential risk factors for vulvovaginal candidiasis in Aligarh, India. Eur J Obstet Gynecol Reprod Biol 2009; 144:68-71. *https://doi.org/10.1016/j.ejogrb.2008.12.020*
20. Olowe OA, Makanjuola OB, *et al*. Prevalence of vulvovaginal candidiasis, trichomoniasis and bacterial vaginosis among pregnant women receiving antenatal care in Southwestern Nigeria. Eur J Microbiol Immunol 2014; 4:193-7. *https://doi.org/10.1556/EUJMI-D-14-00027*
21. Rylander E, Berglund A-L, Krassny C, Petrini B. Vulvovaginal candida in a young sexually active population: prevalence and association with orogenital sex and frequent pain at intercourse. Sex Transm Infect 2004; 80: 54-7. *https://doi.org/10.1136/sti.2003.004192*
22. Sobel JD. Candida vulvovaginitis. Clin Obstet Gynecol 1993; 36:153.
23. Xu J, Schwartz K, *et al*. Effect of antibiotics on vulvovaginal candidiasis: a Metro Net study. J Am Board Fam Med 2008; 21:261-8. *https://doi.org/10.3122/jabfm.2008.04.070169*
24. Spinillo A, Capuzzo E, Nicola S, *et al*. The impact of oral contraception on vulvovaginal candidiasis. Contraception 1995; 51: 293-7. *https://doi.org/10.1016/0010-7824(95)00079-P*
25. Sobe JD, Faro S, Force RW, *et al*. Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations. Am J Obst Gynecol 1998; 178:203-11. *https://doi.org/10.1016/s0002-9378(98)80001-x*
26. Rathod SD, Klausner JD, Krupp K *et al*. Epidemiologic Features of Vulvovaginal Candidiasis among Reproductive-Age Women in India. Infect Dis Obst Gynecol 2012; 2012:859071- 8. *https://doi.org/10.1155/2012/859071*
27. Yadav K, Prakash S. Prevalence of vulvovaginal candidiasis in pregnancy. Glob J Med Sci 2016; 4:108-16.
28. Ttrama JP, Adelson ME, Raphaelli I, *et al*. Detection of Candida species in vaginal samples in a clinical laboratory setting. Infect Dis Obstet Gynecol 2005; 13(2):63-7. *https://doi.org/10.1080/10647440400025629*
29. Bitew A and Abebaw Y. Vulvovaginal candidiasis: species distribution of Candida and their antifungal susceptibility pattern. BMC Women's Health 2018; 18(94): 1-10. *https://doi.org/10.1186/s12905-018-0607-z*
30. Bauters TGM, Dhont MA, Temmerman MI, Nelis HJ. Prevalence of vulvovaginal candidiasis and susceptibility to fluconazole in women. Am J Obstet Gynecol 2002; 187:569-74. https://doi.org/10.1067/mob.2002.125897
31. Haleim MMA, El-Feky EAM, Sayed A, *et al*. Prevalence of non albicans species associated with vulvovaginal candidiasis in Egyptian women. Int J Adv Int Health Sci 2015; 12:304-13.
32. Hedayati MT, Taheri Z, Galinimorhadam T, *et al*. Isolation of different species of candida in patients with vulvovaginal candidiasis from Sari, Iran. Jundishapur J Microbial 2015; 8:e15992. *https://doi.org/10.5812/jjm.8(4)2015.15992*
33. Nyirjesy P, Seeney SM, Grody MHT, *et al*. Chronic fungal vaginitis: the value of cultures. Am J Obstet Gynecol 1995; 173:820-3. *https://doi.org/10.1016/0002-9378(95)90347-X*
34. Sobel JD, Wiesenfeld HC, Martens M, *et al*. Maintenance fluconazole therapy for recurrent vulvovaginal candidiasis. N Engl J Med 2004; 351:876-83. *https://doi.org/10.1056/NEJMoa033114*
35. Hasanvand S, Qomi HA, Kord M, Didehdar M. Molecular epidemiology and *in vitro* antifungal susceptibility of Candida isolates from women with vulvovaginal candidiasis in northern cities of Khuzestan Province. Iran Jundishapur J Microbiol 2017; 10(8):12804. *https://doi.org/10.5812/jjm.12804*